PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of
MULLER ET AL.

Examiner: NOT ASSIGNED

Art Unit: NOT ASSIGNED

Serial No.: **NOT ASSIGNED**

Filed: **FEBRUARY 5, 2002**

Title: **SACCHAROMYCES CEREVISIAE YEAST STRAIN WITH FUNCTIONAL EXPRESSION OF A GLUT TRANSPORTER**

I hereby certify that this correspondence is being deposited with the United States Postal Service as EXPRESS MAIL with EXPRESS MAIL LABEL NO. EL 911210810 US in an envelope addressed to Commissioner for Patents, Washington, D.C. 20231, on

February 5, 2002
Date of Deposit

Dolly Kapadia
Signature

PRELIMINARY AMENDMENT

Commissioner for Patents

Washington, D.C. 20231

Sir:

Please amend the above-identification application as follows:

IN THE SPECIFICATION:

Page 1 below the title, please insert the following paragraph:

FOREIGN PRIORITY CLAIM

This application claims the priority under 35 U.S.C. § 119 of German Application No. 101 06 718.6 filed February 14, 2001, which is hereby incorporated by reference herein in its entirety.

Please remove the Sequence Listing originally filed in this Application and substitute for it the substitute Sequence Listing filed herewith.

IN THE CLAIMS:

Please amend Claims 2-11, 13-17, and 19-25 as follows:

2. (Amended) The strain of the yeast *Saccharomyces cerevisiae* of claim 1 as deposited at the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH as DSM 14035, DSM 14036 or DSM 14037.

3. (Amended) A method for generating a strain of the yeast *Saccharomyces cerevisiae* as claimed in claim 1, comprising the steps of:

a) providing a strain of *Saccharomyces cerevisiae* yeast,

b) eliminating the function of all hexose transporters of the strain of yeast from a) by mutating or deleting the relevant genomic sequences.

4. (Amended) The strain of the yeast *Saccharomyces cerevisiae* as claimed in claim 1, which comprises a GLUT4 gene.

5. (Amended) The strain of the yeast *Saccharomyces cerevisiae* as claimed in claim 4, wherein the GLUT4 gene is under the functional control of a promoter which can be expressed in yeast.

6. (Amended) The strain of the yeast *Saccharomyces cerevisiae* as claimed in claim 4, wherein the Glut4 gene is a human Glut4 gene, a mouse Glut4 gene, or a rat Glut4 gene.

7. (Amended) The strain of the yeast *Saccharomyces cerevisiae* as claimed in claim 4 as deposited at the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH as DSM 14038, DSM 14039 or DSM 14040.

8. (Amended) A method for generating a strain of the yeast *Saccharomyces cerevisiae* as claimed in claim 4, comprising the steps of:

a) providing a strain of the yeast *Saccharomyces cerevisiae* which can no longer grow on substrates with hexoses as the only carbon source, and whose ability of growing on a substrate with a hexose as the only carbon source is restored when a GLUT4 gene is expressed in the strain;

b) transforming the yeast of step a) with a plasmid comprising a GLUT4 gene which is under the functional control of a promoter which can be expressed in yeast;

c) plating the strain of step b) onto a medium comprising glucose as the only carbon source; and

d) isolating the strain that has been plated in accordance with c) and which grows on this medium.

9. (Amended) The method of claim 8, wherein the GLUT4 gene used in transforming step b) is a human GLUT4 gene, a mouse GLUT4 gene, or a rat GLUT4 gene.

10. (Amended) The method of claim 8, wherein a vector with a polynucleotide sequence as shown in SEQ ID No. 9 or 10 is used in transforming step b).

11. (Amended) A method for identifying a compound which increases or reduces the amount of a hexose transported by means of a Glut4 protein, comprising the steps of:

a) providing the strain of claim 4;

b) determining the amount of a hexose taken up by the strain of step a);

c) providing a compound;

d) contacting the strain of step a) with the compound;

e) determining the amount of hexose taken up by the yeast strain after contacting the compound; and

f) identifying a compound as increasing or reducing the amount of hexose transported by means of a Glut4 protein by comparing the amount of hexose taken up by the strain before and after contacting in accordance with d), which is determined in accordance with b) and e).

13. (Amended) A method for treating diabetes or adiposity in a subject comprising administering to the subject an effective amount of a compound which has been identified and, if appropriate, further developed by a method as claimed in claim 11, for the preparation of a pharmaceutical for the treatment of diabetes or adiposity.

14. (Amended) A method for identifying a compound which increases or reduces the amount of a hexose transported by means of a Glut1 protein, comprising the steps of:

a) providing a strain of the yeast *Saccharomyces cerevisiae* which can no longer grow on substrates with hexoses as the only carbon source and whose ability of growing

on a substrate with a hexose as the only carbon source is restored when it expresses a Glut1 gene, this strain comprising a GLUT-1 gene under the functional control of a promoter which can be expressed in yeast;

b) determining the amount of a hexose which is taken up by this strain provided in accordance with a);

c) providing a compound;

d) contacting the strain of the yeast provided in accordance with a) with the compound provided in accordance with c);

e) determining the amount of hexose taken up by the yeast strain after contacting in accordance with d); and

f) identifying a compound as increasing or reducing the amount of a hexose transported by means of a Glut1 protein by comparing the amount of the hexose taken up by the strain before and after contacting in accordance with d), which is determined in accordance with b) and e).

15. (Amended) The method as claimed in claim 14, wherein the strain of step a) has a Strain number of DSM 14026, DSM 14027 or DSM14033 [is provided].

16. (Amended) A pharmaceutical comprising a compound which has been identified and, if appropriate, further developed by the method as claimed in claim 14, and adjuvants for formulating the pharmaceutical for the treatment of diabetes or adiposity.

17. (Amended) A method for treating diabetes or adiposity in a subject, comprising administering to the subject an effective amount of the compound which has been identified and, if appropriate, further developed by the method as claimed in claim 14 for the preparation of a pharmaceutical for the treatment of diabetes or adiposity.

19. (Amended) A method for generating a strain of *Saccharomyces cerevisiae* as claimed in claim 18, comprising the steps of:

a) providing a strain of *Saccharomyces cerevisiae* yeast which can no longer grow on substrates with hexoses as the only carbon source, and whose ability of growing on a substrate with a hexose as the only carbon source is restored when a GLUT4 gene is expressed in this strain;

b) transforming the strain of a) with a plasmid comprising a polynucleotide sequence of SEQ ID No. 13 or 14;

c) plating the strain that has been transformed in accordance with b) onto a medium comprising glucose as the only carbon source; and

d) isolating a strain which has been plated in accordance with c) and which grows on this medium.

20. (Amended) An isolated nucleic acid molecule that encodes a GLUT1 protein having an amino acid sequence in which the valine at position 69 is substituted with methionine.

21. (Amended) The isolated nucleic acid molecule of claim 20, comprising the DNA sequence of SEQ ID NO:13.

22. (Amended) A Glut1 protein encoded by the isolated nucleic acid molecule of claim 21.

23. (Amended) An isolated nucleic acid molecule that encodes a GLUT1 protein having an amino acid sequence in which the valine at position 70 is substituted with methionine.

24. (Amended) The isolated nucleic acid molecule of claim 23, comprising the DNA sequence of SEQ ID NO:14.

25. (Amended) A Glut1 protein encoded by the isolated nucleic acid molecule of claim 24.

REMARKS

Claims 1-25 are presently pending in this case. In this preliminary amendment, Applicants have amended Claims 2-11, 13-17, and 19-25. Applicants have also amended the instant Specification to include a Priority Claim and to submit a substitute Sequence Listing. Support for amended Claims 2-11, 13-17 and 19-25 can be found generally throughout the instant Specification and in Claims 1-25 as filed. Consequently, the instant Amendment introduces no new matter into the instant Application. Attached hereto is a marked-up version of the changes made to the Specification and Claims by the

instant Amendment. The attached page is captioned "**Version With Markings To Show Changes Made.**"

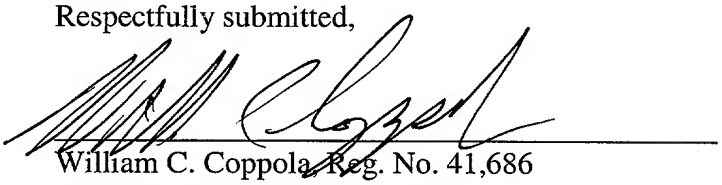
Fees

No fees are believed to be necessitated by the instant response. However, should this be in error, authorization is hereby given to charge Deposit Account no. 18-1982 for any underpayment, or to credit any overpayments.

CONCLUSION

Applicants respectfully submit that the Claims as amended are believed to be in condition for allowance. Thus, early and favorable action on the claims is earnestly solicited.

Respectfully submitted,



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Version With Markings To Show Changes Made

Subject matter that is underlined is to be added, and subject matter that is within brackets is to be removed.

IN THE SPECIFICATION:

Page 1, line 7, please insert the following:

FOREIGN PRIORITY CLAIM

This application claims the priority under 35 U.S.C. § 119 of German Application No. 101 06 718.6 filed February 14, 2001, which is hereby incorporated by reference herein in its entirety.

IN THE CLAIMS:

Please amend Claims 2-11, 13-17, and 19-25 as follows:

2. (Amended) The [A] strain of the yeast *Saccharomyces cerevisiae* of [as claimed in] claim 1 as deposited at the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH as DSM 14035, DSM 14036 or DSM 14037.

3. (Amended) A method for generating [Generation of] a strain of the yeast *Saccharomyces cerevisiae* as claimed in claim 1 [or 2], comprising the steps of: [obtainable by]

c) providing a strain of *Saccharomyces cerevisiae* yeast,

d) eliminating the function of all hexose transporters of the strain of yeast [this yeast] from a) by mutating or deleting the relevant genomic sequences.

4. (Amended) The [A] strain of the yeast *Saccharomyces cerevisiae* as claimed in claim 1 [one or more of claims 1 to 3], which comprises a GLUT4 gene.

5. (Amended) The [A] strain of the yeast *Saccharomyces cerevisiae* as claimed in claim 4, wherein the [a recombinant] GLUT4 gene is under the functional control of a promoter which can be expressed in yeast.

6. (Amended) The [A] strain of the yeast *Saccharomyces cerevisiae* as claimed in claim 4 [or 5], wherein the Glut4 gene is a human Glut4 gene, a mouse Glut4 gene, or a rat Glut4 gene [derived from humans, mice or rats].

7. (Amended) The [A] strain of the yeast *Saccharomyces cerevisiae* as claimed in claim 4 [one or more of claims 4 to 6] as deposited at the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH as DSM 14038, DSM 14039 or DSM 14040.

8. (Amended) A method for generating [The generation of] a strain of the yeast *Saccharomyces cerevisiae* as claimed in claim 4, comprising the steps of: [one or more of claims 4 to 7, which is obtainable by]

a) providing a strain of the yeast *Saccharomyces cerevisiae* which can no longer grow on substrates with hexoses as the only carbon source, and whose ability of growing

on a substrate with a hexose as the only carbon source is restored when a GLUT4 gene is expressed in the strain [Providing a yeast as claimed in any of claims 1 to 3];

b) transforming [Transformation of] the yeast of step a) with [by] a plasmid comprising a GLUT4 gene which is under the functional control of a promoter which can be expressed in yeast;

c) plating the [Plating a] strain of step [which has been transformed in accordance with] b) onto a medium comprising glucose as the only carbon source; and

d) isolating the strain that [Isolating a strain which] has been plated in accordance with c) and which grows on this medium.

9. (Amended) The method of claim 8, wherein the GLUT4 gene used in transforming step b) is a human GLUT4 gene, a mouse GLUT4 gene, or a rat GLUT4 gene [The generation as claimed in claim 8, wherein a GLUT4 gene from humans, mice or rats is used for the transformation].

10. (Amended) The method of claim 8 [generation as claimed in claim 8 or 9], wherein a vector with a polynucleotide sequence as shown in SEQ ID No. 9 or 10 is used in transforming step b) [for the transformation].

11. (Amended) A method [which can be used] for identifying a compound which increases or reduces the amount of a hexose transported by means of a Glut4 protein, comprising the steps of [with the following process steps]:

a) providing the strain of claim 4 [Providing a strain of the yeast *Saccharomyces cerevisiae* as claimed in one or more of claims 4 to 10];

b) determining [Determining] the amount of a hexose [which is] taken up by the strain of step a) [this strain provided in accordance with a)];

c) providing [Providing] a compound;

d) contacting the strain of step [Contacting a strain of the yeast provided in accordance with] a) with the compound [a compound provided in accordance with c)];

e) determining [Determining] the amount of [a] hexose [which is] taken up by [into] the yeast strain after contacting the compound [in accordance with d)]; and

f) [Identifying] identifying a compound as increasing or reducing [which increases or reduces] the amount of [a] hexose transported by means of a Glut4 protein by comparing the amount of [the] hexose taken up by [into] the strain before and after contacting in accordance with d), which is determined in accordance with b) and e).

13. (Amended) A method for treating diabetes or adiposity in a subject comprising administering to the subject an effective amount of a [The use of a] compound which has been identified and, if appropriate, further developed by a method as claimed in claim 11, for the preparation of a pharmaceutical for the treatment of diabetes or adiposity.

14. (Amended) A method [which can be used] for identifying a compound which increases or reduces the amount of a hexose transported by means of a Glut1 protein, comprising the steps of: [with the following process steps:]

a) providing [Providing] a strain of the yeast *Saccharomyces cerevisiae* which can no longer grow on substrates with hexoses as the only carbon source and whose ability of growing on a substrate with a hexose as the only carbon source is restored when it expresses a Glut1 gene, this strain comprising a GLUT-1 gene under the functional control of a promoter which can be expressed in yeast;

b) determining [Determining] the amount of a hexose which is taken up by this strain provided in accordance with a);

c) providing [Providing] a compound;

d) contacting the [Contacting a] strain of the yeast provided in accordance with a) with the [a] compound provided in accordance with c);

e) determining [Determining] the amount of [a] hexose [which is] taken up by [into] the yeast strain after contacting in accordance with d); and

f) [Identifying] identifying a compound as increasing or reducing [which increases or reduces] the amount of a hexose transported by means of a Glut1 protein by comparing the amount of the hexose taken up by [into] the strain before and after contacting in accordance with d), which is determined in accordance with b) and e).

15. (Amended) The [A] method as claimed in claim 14, wherein[, in accordance with a), a] the strain of step a) has a Strain number of [the yeast Saccharomyces cerevisiae with the Strain Number] DSM 14026, DSM 14027 or DSM14033 [is provided].

16. (Amended) A pharmaceutical comprising a compound which has been identified and, if appropriate, further developed by the [a] method as claimed in claim 14 [or 15], and adjuvants for formulating the pharmaceutical for the treatment of diabetes or adiposity.

17. (Amended) A method for treating diabetes or adiposity in a subject, comprising administering to the subject an effective amount of the [The use of a] compound which has been identified and, if appropriate, further developed by the [a] method as claimed in claim 14 [or 15,] for the preparation of a pharmaceutical for the treatment of diabetes or adiposity.

19. (Amended) A method for generating [The generation of] a strain of *Saccharomyces cerevisiae* as claimed in claim 18, comprising the steps of: [which is obtainable by]

a) providing [Providing] a strain of *Saccharomyces cerevisiae* yeast which can no longer grow on substrates with hexoses as the only carbon source, and whose ability of growing on a substrate with a hexose as the only carbon source is restored when a GLUT4 gene is expressed in this strain [yeast as claimed in any of claims 1 to 3];

b) transforming [Transformation of] the strain [yeast] of a) with a plasmid comprising a polynucleotide sequence of SEQ ID No. 13 or 14;

c) plating the strain that [Plating a strain which] has been transformed in accordance with b) onto a medium comprising glucose as the only carbon source; and

d) isolating [Isolating] a strain which has been plated in accordance with c) and which grows on this medium.

20. (Amended) An isolated nucleic acid molecule that encodes [A polynucleotide sequence encoding] a GLUT1 protein having an amino acid sequence in which the [with a substitution of] valine at position 69 is substituted with methionine [at position 69 of the amino acid sequence].

21. (Amended) The isolated nucleic acid molecule of [A polynucleotide sequence as claimed in] claim 20, comprising [a] the DNA sequence of SEQ ID NO:[Nr.] 13.

22. (Amended) A Glut1 protein encoded by the isolated nucleic acid molecule of [a polynucleotide sequence as claimed in] claim [20 or] 21.

23. (Amended) An isolated nucleic acid molecule that encodes [A polynucleotide sequence encoding] a GLUT1 protein having an amino acid sequence in which the [with a substitution of] valine at position 70 is substituted with methionine [at position 70 of the amino acid sequence].

24. (Amended) The isolated nucleic acid molecule of [A polynucleotide sequence as claimed in] claim 23, comprising the DNA [a] sequence of SEQ ID NO: [Nr.] 14.

25. (Amended) A Glut1 protein encoded by the isolated nucleic acid molecule of [a polynucleotide sequence as claimed in] claim [23 or] 24.